Utilization and Toxicity of Peptonized Iron and Ferrous Sulfate

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RECENT reports of the fatal poisoning of several children by the accidental ingestion of tablets of ferrous sulfate inspired a search for another less toxic iron product which would still be effective in the regeneration of hemoglobin. Experience with peptonized iron suggested that it could be an appropriate choice.

A preparation composed of powdered iron oxide in oil, whey, vinegar, cow's urine, and milk was the form of peptonized iron used by the ancient Hindus.[1] An improved preparation of peptonized iron composed of iron oxide, peptone, and sodium citrate became official in the fourth edition (1916) of the *National Formulary* but was deleted from the tenth edition (1955). The difficulty of tableting and the excessive cost of peptonized iron as compared to ferrous sulfate was the principal reason for its decline as a hematinic. The U. S. Dispensatory (Twenty-first Edition) states that the reason for preferring iron peptonate over inorganic salts of iron is "the absence of injurious effects upon the teeth and mucous membrane and its deficiency of astringency."

IRON UTILIZATION

The fact that peptonized iron does not ionize in solution suggests that its utilization by the body might be poor. The significance of this statement is underlined by the failure of the α,α' -dipyridyl test to show the presence of iron in a solution of peptonized iron. The dipyridyl test is used to determine the presence of available iron in a foodstuff.[2] These properties indicate the need for determining to what extent the iron in the peptonized form is assimilated.

Method in Anemic Rats

Male rates, 250–300 g, were placed on an iron-free diet for two weeks. This consisted of powdered skim milk and 0.5 per cent peanut oil made to a pasty consistency with distilled water. They were fasted for from 18 to 24 hours, anesthetized with ether, and made anemic by cardiac puncture in an amount equivalent to 2.5 per cent of body weight. Ephedrine sulfate, 20 mg/kg, was given subcutaneously a few minutes before bleeding to counteract the shock produced by the rapid removal of so much blood, but artificial respiration was often necessary after bleeding the animals. A 23-gauge, short-bevel, half-inch needle was found to be most satisfactory.

Hemoglobin was determined with a Sahli-Haden hemoglobinometer four days after bleeding, and, if the hemoglobin level was above 10 g, the animals were not used.

Peptonized iron and ferrous sulfate were administered through an oral feeding tube five days a week for 16 days. The iron compounds were given at two daily dosage levels: 0.3 mg and 0.5 mg per rat. Copper in the form of copper chloride was added to the distilled drinking water at 2 mg/100 ml as an element necessary for iron absorption in rats.[3]

Results in Anemic Rats

The hemoglobin level was determined again after 16 days of controlled iron intake, and the results of this procedure are seen in Table I. (The iron content of ferrous sulfate was calculated to be 20 per cent and that of peptonized iron, 16.9 per cent.)

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TABLE I

Hemoglobin Response to Peptonized Iron and Ferrous Sulfate in Anemic Rats

	No. of animals	Dosage of Fe/ rat (mg)	Initial Hb level	Hb level after bleeding	Hb level 16 days
Peptonized iron	20	0.5	14.2	10	16.4
Ferrous sulfate	20	0.5	14.2	9.8	16.2
Peptonized iron	10	0.3	14.2	9.9	16.2
Ferrous sulfate	10	0.3	14.2	9.8	13
Control	20	—	14.2	10	12

Hb—hemoglobin in g/100 ml.

The hemoglobin test used was not accurate enough to make possible a statement of the absolute ratio of absorption and utilization, but the results give an indication of the degree of action and show that peptonized iron regenerates hemoglobin in anemic rats as fast as ferrous sulfate.

It does not seem unusual to find that peptonized iron is readily assimilated, for Will and Vilter,[4] using labeled iron, found that the very stable iron sodium ethylene diamine tetraacetate was broken down and the iron absorbed.

Method in Gastrectomized Rats

Peptonized iron liberates free iron in the presence of hydrochloric acid, which suggests that the iron might not be available in achlorhydria. To test the effect of achlorhydria, rats were gastrectomized. This experiment was carried out as in the previous study, with one exception: the rats were gastrectomized the day after bleeding and five days were allowed for recovery. This group did not respond to the same amount of iron as did the animals in the previous study, so ten times the dosage was used (5 mg iron per rat per day).

TABLE II

Hemoglobin Response of Anemic, Gastrectomized Rats to Peptonized Iron and to Ferrous Sulfate

	No. of animals	Hb Level after bleeding and gastrectomy	Dosage of Fe/rat *mg*	Hb level 16 days
Peptonized iron	3	8.6	5	13
Ferrous sulfate	3	9	5	14.2
Control (no iron)	3	6.4	0	Died

Results in Gastrectomized Rats

Two of the control animals died of respiratory infections and the remaining animal had a hemoglobin level below 4 g. This animal also died shortly after beginning the second period. The death of the control animals only would indicate that the anemic state was a contributory cause. It would be illogical to assume that the gastrectomized rats on an iron-free diet would return to a normal hemoglobin level when intact rats on the same diet failed to make a complete hemoglobin recovery.

The failure of these preparations to regenerate hemoglobin to the same degree as in non-gastrectomized rats would indicate that hydrochloric acid is required for maximum absorption of both peptonized iron and ferrous sulfate.

That the hemoglobin can be returned to normal by these preparations, even though tenfold doses are required, shows that hydrochloric acid is not indispensable for iron utilization. This fact is essentially what Barer and Fowler[4] found in studying the absorption of several iron compounds in humans.

TOXICITY OF IRON

Data on the acute toxicity of peptonized iron are as scanty as those on its utilization; to supplement the meager information in this regard, the acute toxicity of peptonized iron was compared with that of ferrous sulfate in both rats and rabbits.

Acute Oral Toxicity in Rats

The acute toxicity of these iron compounds was determined in albino rats of mixed sexes weighing 250–400 g. The compounds were

given orally dissolved in water at 200 mg/ml. The aminals were observed for two weeks. The double integration method[6] was used to callate the LD_{50}.

TABLE III
Acute Oral Toxicity of Peptonized Iron and Ferrous Sulfate in Rats

	LD_{50}		
	No. of animals	as salt *mg/kg*	As iron *mg/kg*
Peptonized iron	60	6370	1077
Ferrous sulfate	40	1720	344

The acute oral LD_{50} as shown in this table indicates that, based on iron contents, ferrous sulfate is three times as toxic as peptonized iron. Death from peptonized iron occurred mostly between 9 and 18 hours after administration, while that from ferrous sulfate was usually fatal within 1 to 5 hours. Both compounds produced diarrhea and depression, the intensity of which increased with the dose. The animals killed by peptonized iron did not convulse at the time of death as did those killed by ferrous sulfate.

Acute Oral Toxicity in Rabbits

The LD_{50} of each of these iron compounds was also compared in albino rabbits of mixed sex weighing from 2 to 3 kg. The procedure used was the same as that used on rats. The results are given in Table IV.

TABLE IV
Acute Oral Toxicity of Peptonized Iron and Ferrous Sulfate in Rabbits

	LD_{50}		
	No. of animals	As salt (*mg/kg*)	As iron (*mg/kg*)
Peptonized iron	31	3625	612
Ferrous sulfate	34	1028	205.6

Acute Oral Toxicity in Dogs

The iron salts were mixed with powdered milk in the form of a paste. The dogs were not fed for the previous 24 hours, which made for ready consumption of the mixture. However none of the dogs died because the emetic threshold in dogs is reached before a fatal dose of these iron compounds can be absorbed.

TABLE V
Emetic Response to Peptonized Iron and Ferrous Sulfate in Dogs

	Amount		No. vomiting
	As salt *mg/kg*	As iron *mg/kg*	No. used
Peptonized iron	2000	339	0/4
	2500	423	1/4
	3000	507	1/4
	4000	676	4/4
Ferrous sulfate	1000	200	4/4

The results of these tests are given in Table V. It shows that peptonized iron caused vomiting in all four dogs in doses approaching 4 g/kg, as compared with 1 g/kg for ferrous sulfate: again we see toxic reactions in multiples of three based on iron content. In animals which did not vomit, copious diarrhea resulted, but no other toxic symptoms were observed. It is interesting to note that vomiting began in dogs at doses of 2.5 g/kg of peptonized iron, which is also the lowest dose of this substance which was lethal to rabbits.

It might appear that a difference 3 to 1 in *oral* toxicity of these two iron compounds in rats, rabbits, and dogs would be an expression of the degree and rate of absorption. This, however, is not true; Nissim[7] in comparing the *intravenous* toxicity of these preparations in mice found the LD_{50} for ferrous sulfate to be 11 mg/kg (as ferrous ion) and for peptonized iron to be 33 mg/kg (as ferric ion).

Local Toxicity

A common test for local toxicity is the intravenous injection into an animal of a dye, such as Evans or trypan blue: where local irritation occurs, capillary permeability is increased and the dye leaks out of the vascular system, forming a conspicuous blue stain in the irritated tissues. No such stain or irritation is seen in the stomach of rats which have received fatal oral doses of peptonized iron; but a great deal of irritation is observed by

this method in the stomachs of rats which have received fatal oral doses of ferrous sulfate.

A more accurate comparison of local irritation is obtained by comparing the minimum concentrations which will cause sloughing when injected intradermally. Intradermal injections of 0.1 ml of 10 per cent peptonized iron solution did not cause sloughing in rabbits, but 0.1 ml of 2 per cent ferrous sulfate solution caused sloughing.

SUMMARY

Peptonized iron is effectively utilized as a hematinic in rats.

The oral LD_{50} for peptonized iron is one third that of ferrous sulfate in rats and rabbits. This same ratio is seen in the emetic response of dogs to peptonized iron and ferrous sulfate.

More than five times as much peptonized iron, injected intradermally, was required to produce local irritation as compared to ferrous sulfate.

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